PENDING CLAIMS (as of November 26, 2002)

1. A recombinant allergen, characterised in that it is a mutant of a naturally occurring allergen, wherein the mutant allergen has at least four primary mutations, which each reduce the specific IgE binding capability of the mutated allergen as compared to the IgE binding capability of the said naturally occurring allergen, wherein each primary mutation is a substitution of one surface-exposed amino acid residue with another residue, which does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic species from which said naturally occurring allergen originates, wherein each primary mutation is spaced from each other primary mutation by at least 15 Å, and wherein the primary mutations are placed in such a manner that at least one circular surface region with a area of 800 $Å^2$ comprises no mutation.

2. (Amended) A recombinant allergen according to claim 1, wherein the primary mutations are spaced between about 20 to 30 Å.

3. (Amended) A recombinant allergen according to claim 1 comprising a number of secondary mutations, which each reduce the specific IgE binding capability of the mutated allergen as compared to the binding capability of the said naturally occurring allergen, wherein each secondary mutation is a substitution of one surface-exposed amino acid residue with another residue, which does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic species from which said naturally occurring allergen originates, wherein the secondary mutations are placed outside the said circular region.

4. (Amended) A recombinant allergen according to claim 1, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen has a solvent accessibility of above 20 %.

5. (Amended) A recombinant allergen according to claim 1, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen is

conserved with more than 70 % identity in all known homologous proteins within the species from which said naturally occurring allergen originates.

6. (Amended) A recombinant allergen according to claim 1, which essentially has the same α-carbon backbone tertiary structure as said naturally occurring allergen.

7. (Amended) A recombinant allergen according to claim 1, wherein each amino acid residue to be incorporated into the mutant allergen does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic genus from which said naturally occurring allergen originates.

8. (Amended) A recombinant allergen according claim 1, characterised in that the specific IgE binding to the mutated allergen is reduced by at least 5%.

9. A recombinant allergen according to claim 6, characterised in that when comparing the α-carbon backbone tertiary structures of the mutant and the naturally occurring allergen molecules, the average root mean square deviation of the atomic coordinates is below 2Å.

10. (Amended) A recombinant allergen according to claim 1, characterised in that said circular surface region comprises atoms of 15-25 amino acid residues.

11. (Amended) A recombinant allergen according to claim 1, characterised in that the surface-exposed amino acid residues are ranked with respect to solvent accessibility, and that one or more amino acids among the more solvent accessible ones are substituted.

12. (Amended) A recombinant allergen according to claim 1, characterised in that the surface-exposed amino acid residues are ranked with respect to degree of conservation in all known homologous proteins within the species from which said naturally occurring allergen originates, and that one or more amino acids among the more conserved ones are substituted.

13. (Amended) A recombinant allergen according to claim 1, wherein the mutant allergen is a non-naturally occurring allergen.

14. (Amended) A recombinant allergen according to claim 1 comprising from 5 to 20 primary mutations.

15. (Amended) A recombinant allergen according to claim 3 characterised in that the mutant allergen comprises from 1 to 4 secondary mutations per primary mutation.

16. (Amended) A recombinant allergen according to claim 1, characterised in that one or more of the substitutions is carried out by site-directed mutagenesis.

17. (Amended) A recombinant allergen according to claim 1, characterised in that one or more of the substitutions is carried out by DNA shuffling.

18. (Amended) A recombinant allergen according to claim 1, characterised in that it is a mutant of an inhalation allergen.

19. A recombinant allergen according to claim 18, characterised in that it is a mutant of a pollen allergen.

20. A recombinant allergen according to claim 19 characterised in that it is a mutant of a pollen allergen originating from the taxonomic order of *Fagales*, *Oleales* or *Pinales*.

21. A recombinant allergen according to claim 20, characterised in that it is a mutant of Bet v 1.

22. A recombinant allergen according to claim 21, characterised in that one or more of the substitutions is selected from the group consisting of V2, D72, E87, K-129, E-60, N--7, K-65, P-108, N-159, D-93, K-123, K-32, D-125, R-145, D-109, E-127, Q-36, E-131, L-152, E-6, E-96, D-156, P-63, H-76, E-8, K-134, E-45, T-10, V-12, K-20, S-155, H-126, P-50, N-

78, K-119, V-2, L-24, E-42, N-4, A-153, I-44, E-138, G-61, A-130, R-70, N-28, P-35, S-149, K-103, Y-150, H-154, N-43, A-106, K-115, P-14, Y-5, K-137, E-141, E-87 and E-73.

23. A recombinant allergen according to claim 19, characterised in that it is a mutant of a pollen allergen originating from the taxonomic order of *Poales*.

24. A recombinant allergen according to claim 19, characterised in that it is a mutant of a pollen allergen originating from the taxonomic order of *Asterales* or *Urticales*.

25. A recombinant allergen according to claim 18, characterised in that it is a mutant of a house dust mite allergen.

26. A recombinant allergen according to claim 25, characterised in that it is a mutant of a mite allergen originating from *Dermatophagoides*.

27. A recombinant allergen according to claim 18, characterised in that it is a mutant of a cockroach allergen.

28. A recombinant allergen according to claim 18, characterised in that it is a mutant of an animal allergen.

29. A recombinant allergen according to claim 28, characterised in that it is a mutant of an animal allergen originating from cat, dog or horse.

30. (Amended) A recombinant allergen according to claim 1 characterised in that it is a mutant of a venom allergen.

31. A recombinant allergen according to claim 30, characterised in that it is a mutant of a venom allergen originating from the taxonomic order of *Hymenoptera*.

32. A recombinant allergen according to claim 31, characterised in that is a mutant of a venom allergen from the taxonomic order of *Vespidae*, *Apidae* and *Formicoidae*.

33. (Amended) A recombinant allergen according to claim 30 characterised in that it is a mutant of Ves v 5.

34. A recombinant allergen according to claim 33 characterised in that one or more of the substitutions is selected from the group consisting of K-16, K-185, K-11, K-44, K-210, R-63, K-13, F-6, K-149, K-128, E-184, K-112, F-157, E-3, K-29, N-203, N-34, K-78, K-151, L-15, L-158, Y-102, W-186, K-134, D-87, K-52, T-67, T-125, K-150, Y-40, Q-48, L-65, K-81, Q-101, Q-208, K-144, N-8, N-70, H-104, Q-45, K-137, K-159, E-205, N-82, A-111, D-131, K-24, [--]V-36, N-7, M-138, T-209, V-84, K-172, V-19, D-56, P-73, G-33, T-106, N-170, L-28, T-43, Q-114, C-10, K-60, N-31, K-47, E-5, D-145, V-38, A-127, D-156, E-204, P-71, G-26, Y-129, D-141, F-201, R-68, N-200, D-49, S-153, K-35, S-39, Y-25, V-37, G-18, W-85 and I-182.

35. (Amended) A pharmaceutical composition comprising the recombinant allergen according to claim 1 and at least one of a pharmaceutically acceptable carrier, excipient, or adjuvant.

37. (Amended) A composition comprising two or more recombinant mutant allergen variants according to claim 1, wherein each variant is defined by having at least one primary mutation, which is absent in at least one of the other variants, wherein for each variant no secondary mutation is present within a radius of 15 Å from each absent primary mutation.

38. (Amended) A composition according to claim 37 comprising 2-12 variants.

39. (Amended) A composition according to claim 37 further comprising at least one of a pharmaceutically acceptable carrier, excipient, or adjuvant.

43. (Amended) A method of generating an immune response in a subject comprising administering to the subject a recombinant allergen according to claim 1 or a composition according to any one of claims 35, 37 or 39.

44. (Amended) A method of vaccinating a subject comprising administering to the subject a recombinant allergen according to claim 1 or a composition according to any one of claims 35, 37 or 39.

47. (Amended) A method for the treatment, prevention or alleviation of allergic reactions in a subject comprising administering to a subject a recombinant allergen according to claim 1 or a composition according to any one of claims 35, 37 or 39.

48. (Amended) A method of preparing a recombinant allergen according to claim 1, comprising

a) identifying a number of amino acid residues in a naturally occurring allergen, which have a solvent accessibility of at least 20%;

b) selecting at least four of the identified amino acid residues in such a manner that each selected amino acid is spaced from each other selected amino acid by at least 15 Å, and that the selected amino acids are placed in such a manner that at least one circular surface region with an area of 800 $Å^2$ comprises no selected amino acid; and

c) effecting for each of the selected amino acids a primary mutation, which reduces the specific IgE binding capability of the mutated allergen as compared to the binding capability of the said naturally occurring allergen, wherein each primary mutation is a substitution of a selected amino acid residue with another amino acid, which does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic species from which said naturally occurring allergen originates.

49. (Amended) A method according to claim 48, characterised in ranking said identified amino acid residues with respect to solvent accessibility and substituting one or more amino acids among the more solvent accessible ones.

50. (Amended) A method according to claim 48, characterised in selecting identified amino acid residues, which are conserved with more than 70 % identity in all known homologous proteins within the species from which said naturally occurring allergen originates.

51. (Amended) A method according to claim 50, characterised in ranking said identified amino acid residues with respect to degree of conservation in all known homologous proteins within the species from which said naturally occurring allergen originates and substituting one or more amino acids among the more conserved ones.

52. (Amended) A method according to claim 48 comprising selecting the identified amino acids so as to form a mutant allergen, which has essentially the same α-carbon backbone tertiary structure as said naturally occurring allergen.

53. (Amended) A method according to claim 48 characterised in that the substitution of amino acid residues is carried out by site-directed mutagenesis.

54. (Amended) A method of preparing a recombinant allergen according to claim 1 comprising DNA shuffling (molecular breeding) of the DNA encoding the corresponding naturally occurring allergen to produce said recombinant allergen.

55. (Amended) A DNA sequence encoding a recombinant allergen according to claim 1, a derivative thereof, a partial sequence thereof, a degenerated sequence thereof or a sequence, which hybridises thereto under stringent conditions, wherein said derivative, partial sequence, degenerated sequence or hybridising sequence encodes a peptide having at least one B cell epitope.

56. A DNA sequence according to claim 55, which is a derivative of the DNA sequence encoding the naturally occurring allergen.

57. A DNA sequence according to claim 56, wherein the derivative is obtained by site-directed mutagenesis of the DNA encoding the naturally occurring allergen.

58. (Amended) A DNA sequence according to claim 56, wherein the sequence is a derivative of the sequence shown in Fig. 3, wherein the DNA sequence encodes an allergen

having at least four mutations selected from the group consisting of V2, D72, E87, K-129, E-60, N-47, K-65, P-108, N-159, D-93, K-123, K-32, D-125, R-145, D-109, E-127, Q-36, E-131, L-152, E-6, E-96, D-156, P-63, H-76, E-8, K-134, E-45, T-10, V-12, K-20, S-155, H-126, P-50, N-78, K-119, V-2, L-24, E-42, N-4, A-153, I-44, E-138, G-61, A-130, R-70, N-28, P-35, S-149, K-103, Y-150, H-154, N-43, A-106, K-115, P-14, Y-5, K-137, E-141, E-87 and E-73.

59. (Amended) A DNA sequence according to claim 56, wherein the sequence is a derivative of the sequence shown in Fig. 13, wherein the DNA sequence encodes an allergen having at least four mutations selected from the group consisting of K-16, K-185, K-11, K-44, K-210, R-63, K-13, F-6, K-149, K-128, E-184, K-112, F-157, E-3, K-29, N-203, N-34, K-78, K-151, L-15, L-158, Y-102, W-186, K-134, D-87, K-52, T-67, T-125, K-150, Y-40, Q-48, L-65, K-81, Q-101, Q-208, K-144, N-8, N-70, H-104, Q-45, K-137, K-159, E-205, N-82, A-111, D-131, K-24, V-36, N-7, M-138, T-209, V-84, K-172, V-19, D-56, P-73, G-33, T-106, N-170, L-28, T-43, Q-114, C-10, K-60, N-31, K-47, E-5, D-145, V-38, A-127, D-156, E-204, P-71, G-26, Y-129, D-141, F-201, R-68, N-200, D-49, S-153, K-35, S-39, Y-25, V-37, G-18, W-85 and I-182.

60. (Amended) A DNA sequence according to claim 56, wherein the sequence is a derivative of the sequence shown in Fig. 16, wherein the DNA sequence encodes an allergen having at least four mutations selected from the group consisting of R-128, D-129, H-11, H-30, S-1, K-77, Y-75, R-31, K-82, K-6, K-96, K-48, K-55, K-89, Q-85, W-92, I-97, H-22, V-65, S-24, H-74, K-126, L-61, P-26, N-93, D-64, I-28, K-14, K-100, E-62, I-127, E-102, E-25, P-66, L-17, G-60, P-95, E-53, V-81, K-51, N-103, Q-2, N-46, E-42, T-91, D-87, N-10, M-111, C-8, H-124, I-68, P-79, K-109 and R-128, D-129, H-11, H-30, S-1, K-77, Y-75, R-31, K-82, K-6, K-96, K-48, K-55, K-89, Q-85, W-92, I-97, H-22, V-65, S-24, H-74, K-126, L-61, P-26, N-93, D-64, I-28, K-14, K-100, E-62, I-127, E-102, E-25, P-66, L-17, G-60, P-95, E-53, V-81, K-51, N-103, Q-2, N-46, E-42, T-91, D-87, N-10, M-111, C-8, H-124, I-68, P-79, K-109 and K-15.

61. (Amended) An expression vector comprising the DNA according to any one of claims 55-60 operably linked to a promoter.

62. A host cell comprising the expression vector of claim 61.

63. A method of producing a recombinant mutant allergen comprising the step of cultivating the host cell according to claim 62.

64. (Amended) A recombinant allergen according to claim 1 comprising at least one T cell epitope capable of stimulating a T cell clone or T cell line specific for the naturally occurring allergen.

65. (Amended) A diagnostic assay for assessing relevance, safety or outcome of therapy of a subject using a recombinant mutant allergen according to claim 1, comprising assessing the level of reactivity between IgE in a sample from said subject and said mutant allergen.

66. The recombinant allergen of claim 2 wherein the primary mutations are spaced by at least 25 Å.

67. The recombinant allergen of claim 66 wherein the primary mutations are spaced by at least 30 Å.

68. The recombinant allergen according to claim 4, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen has a solvent accessibility of above 30 %.

69. The recombinant allergen according to claim 68, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen has a solvent accessibility of above 40 %.

70. The recombinant allergen according to claim 69, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen has a solvent accessibility of above 50 %.

71. A recombinant allergen according to claim 5, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen is conserved with more than 80 % identity in all known homologous proteins within the species from which said naturally occurring allergen originates.

72. A recombinant allergen according to claim 71, wherein at least one of the surface-exposed amino acids to be substituted in the naturally occurring allergen is conserved with more than 90 % identity in all known homologous proteins within the species from which said naturally occurring allergen originates.

73. A recombinant allergen according to claim 7, wherein each amino acid residue to be incorporated into the mutant allergen does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic subfamily from which said naturally occurring allergen originates.

74. A recombinant allergen according to claim 73, wherein each amino acid residue to be incorporated into the mutant allergen does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic family from which said naturally occurring allergen originates.

75. A recombinant allergen according to claim 74, wherein each amino acid residue to be incorporated into the mutant allergen does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic superfamily from which said naturally occurring allergen originates.

76. A recombinant allergen according to claim 75, wherein each amino acid residue to be incorporated into the mutant allergen does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic legion from which said naturally occurring allergen originates.

77. A recombinant allergen according to claim 76, wherein each amino acid residue to be incorporated into the mutant allergen does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic suborder from which said naturally occurring allergen originates.

78. A recombinant allergen according to claim 77, wherein each amino acid residue to be incorporated into the mutant allergen does not occur in the same position in the amino acid sequence of any known homologous protein within the taxonomic order from which said naturally occurring allergen originates.

79. A recombinant allergen according claim 8, characterised in that the specific IgE binding to the mutated allergen is reduced by at least 10%.

80. A recombinant allergen according to claim 14 comprising from 6 to 15 primary mutations.

81. A recombinant allergen according to claim 80 comprising from 7 to 12 primary mutations.

82. A recombinant allergen according to claim 81 comprising from 8 to 10 primary mutations.

83. A composition according to claim 38 comprising 3-10 variants.

84. A composition according to claim 83 comprising 4-8 variants.

85. A composition according to claim 84 comprising 5-7 variants.